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Modulation of gene expression in multiple hematopoietic cell lineages following retroviral vector gene transfer

(stem cells/gene regulation)

MARIA-CRISTINA MAGLI*†‡, JOHN E. DICK§¶‖, DENNIS HUSZAR§¶, ALAN BERNSTEIN§¶, AND ROBERT A. PHILLIPS*†**

Departments of *Medical Biophysics and §Medical Genetics, University of Toronto, and †Division of Haematology and Oncology, Hospital for Sick Children, 555 University Avenue, Toronto, ON, M5G 1X8, Canada; and ¶Division of Molecular and Developmental Biology, Mt. Sinai Hospital Research Institute, 600 University Avenue, Toronto, ON, M5G 1X5, Canada

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ABSTRACT **Retrovirus vectors offer a simple and highly efficient method for introducing new genes into mammalian cells. Here, we have examined the efficiency of gene transfer into hematopoietic cells with retrovirus vectors carrying the neomycin (*neo*) resistance gene expressed from different transcriptional regulatory regions. Direct infection of mouse bone marrow cells resulted in high efficiencies of gene transfer into a variety of myeloid progenitor cells, including pluripotent, erythroid, and granulocyte–macrophage colony-forming cells with all the vectors examined. However, the progeny derived from individual pluripotent progenitor cells infected with different vectors differed markedly in the proportion of G418-resistant progenitor cells, as judged by their ability to survive selection in the drug G418. This biological assay suggests that the highest level of expression was observed when the *neo* gene was expressed from constructs that contained the herpes thymidine kinase promoter rather than the viral long terminal repeat or the simian virus 40 early region promoter. In contrast, *neo* gene expression was highest in fibroblasts infected with the vector containing the simian virus 40 early region promoter. These results show that high and sustainable levels of gene expression in hematopoietic cells can be obtained with retrovirus vectors containing appropriate transcriptional regulatory regions.**

The hematopoietic system is composed of a hierarchy of cells that range from fully differentiated nonproliferating cells to pluripotent stem cells with extensive capacity to self-renew, proliferate, and differentiate (1). Fully differentiated mature cells are produced by progenitors committed to a particular lineage. These committed progenitors, as well as multipotent progenitors capable of producing several different cell types, can be assayed by *in vitro* and *in vivo* colony assays (2). The description of methods for introducing new genes into these progenitor cells provides a useful approach to understanding the complex genetic events associated with differentiation (3). The ability to transfer entire genes or regulatory elements into fibroblast cell lines, for example, has led to a dramatic increase in our knowledge of gene regulation and expression. Using various hematopoietic colony assays, the expression of lineage-specific genes such as the genes for hemoglobin, immunoglobulin, hematopoietic growth factors, or several cellular oncogenes implicated in hematopoiesis can be investigated in detail for their effects on differentiation, proliferation, and self-renewal.

Retrovirus vectors provide the most efficient method for introducing new genetic information into various hematopoietic progenitors. These vectors appear able to infect all classes of hematopoietic cells (4–11). However, for these vectors to be useful as vehicles for carrying new genetic information into cells, it is essential that expression of newly introduced genes be appropriately regulated. Since progenitor cells make up a low proportion of hematopoietic tissues, we have indirectly analyzed gene expression in these cells by measuring their ability to grow in the presence of G418. Using this biological assay we have analyzed the levels of *neo* gene expression in hematopoietic progenitor cells infected with retrovirus vectors containing different transcriptional enhancer sequences or promoter regions. Expression of the *neo* gene was sufficiently high to confer resistance to toxic concentrations of the neomycin analogue G418 immediately after infection. Clonal analysis of the pluripotent, erythroid, and granulocyte/macrophage progenitors derived from individual spleen colony-forming units (CFU-S) infected with different neo vectors demonstrated large differences in the proportion of progenitor cells able to form colonies *in vitro* in the presence of G418. These results suggest that sustained levels of gene expression can be obtained in cells of the hematopoietic system by using appropriate transcriptional regulatory regions.

MATERIALS AND METHODS

Mice. BALB/c and CBA/J mice were purchased from The Jackson Laboratory. They were housed in the animal facility at the Ontario Cancer Institute and given food and water ad libitum. In some experiments, mice to be used as recipients received 9.0-9.5 Gy of whole body radiation prior to intravenous injection of cells.

***In Vitro* Colony Assays.** Erythroid burst-forming units (BFU-E), granulocyte–macrophage colony-forming units (CFU-GM), and multipotent colony-forming units (CFU-MIX) were measured with standard colony assays in methyl cellulose. The culture conditions have been described in detail elsewhere (12). Briefly, 10^5–10^6 bone marrow cells were cultured in 1–3 ml of Iscove's modified Dulbecco's medium (IMDM) containing 1-thioglycerol, methyl cellulose, bovine serum albumin, 5% (vol/vol) fetal calf serum, iron-saturated transferrin, lecithin, oleic acid, cholesterol, human urinary erythropoietin (0.5–1 unit/ml), 10% (vol/vol) conditioned medium from WEHI-3B (D^-) cells, and 10% (vol/vol)

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Abbreviations: CFU-S, spleen colony-forming unit(s); BFU-E, erythroid burst-forming unit(s); CFU-GM, granulocyte–macrophage colony-forming unit(s); CFU-MIX, multipotent colony-forming unit(s); LTR, long terminal repeat; SV40, simian virus 40; HSV, herpes simplex virus; Mo-MuLV, Moloney murine leukemia virus; $G418^R$, G418-resistant.

‡Present address: Istituto Internazionale Genetica e Biofisica, C.P. 3061, 80100, Napoli, Italia.
‖Present address: Dept. of Genetics, Research Institute Hospital for Sick Children, Toronto, Canada.
**To whom reprint requests should be addressed.

conditioned medium from 5637 cells as source of colony-stimulating activities. Cultures were scored at 10–14 days for CFU-GM, BFU-E, and CFU-MIX colony types.

Spleen Colony Assay. Following infection bone marrow cells were injected into lethally irradiated (9 Gy) CBA/J mice (13). Small numbers of cells (2–5 × 10^4 cells) were injected to generate individual spleen colonies that were analyzed for drug resistance in methyl cellulose cultures. Spleen colonies were dissected out and dispersed into single cell suspensions in IMDM containing 50% (vol/vol) fetal calf serum, and 10^4–10^6 cells were then plated in semisolid medium in the presence or absence of G418.

Retrovirus Vectors. The vectors used in these experiments are summarized in Fig. 1. All of the vectors used in this study were derived from Moloney murine leukemia virus (Mo-MuLV). Details of the construction of the NEOμ, N2, MLV-NEO.1, and SV(X) vectors have been described (7, 8, 14, 15). The NEOμ vector contains the immunoglobulin heavy chain enhancer cloned into SV(X). The MoTN vector was constructed by cloning the 237-base-pair (bp) *Pst* I fragment carrying the herpes virus *tk* promoter into pUC8 to provide *Bam*HI and *Hin*dIII restriction enzyme sites. This *Bam*HI–*Hin*dIII fragment was cloned together with the 1.5-kilobase (kb) *Hin*dIII–*Bam*HI *neo* fragment from Tn*5* into a *Bam*HI/*Bgl* II-digested pBR322 plasmid containing the Mo-MuLV genome. The NeoF101 vector was constructed by attaching *Bam*HI linkers to the 132-bp *Pvu* II fragment 4 from the F101 mutant polyoma virus (16) and cloning this *Bam*HI fragment into the *Bam*HI site of SV(X). The *Pvu* II fragment 4 carries the F101 enhancer that allows polyoma virus to replicate in embryonal carcinoma cells (16). These vectors were all transfected into ψ_2 helper cells (17). To obtain cell lines producing amphotropic pseudotyped vector particles, PA12 amphotropic helper cells (18) were mixed with ψ_2 cells and grown together. Unexpectedly, the MLV-NEO.1-infected culture of ψ_2 and PA12 cells produced replication competent helper virus in addition to neo virus. Similar findings have been reported by Miller *et al.* (19).

Neomycin Phosphotransferase Assay. Neomycin phosphotransferase was assayed essentially as described by Reiss *et al.* (20). Cell lysates were electrophoresed in a nondenaturing polyacrylamide gel that was then overlaid with an agarose gel containing kanamycin sulfate at 25 μg/ml and 300 μCi (2 nM) of [γ-^{32}P]ATP (1 Ci = 37 GBq). Following 30 min of incubation at room temperature the agarose gel was blotted with Whatman P81 paper. The P81 paper was subsequently washed as described (20), dried, and exposed to x-ray film.

Method of Infection. Transfer and expression of a heterologous gene with a retrovirus into cells as rare as hemopoietic progenitors requires an efficient infection protocol. Several parameters were examined; those listed below had the greatest effects on the infection frequency.

(*i*) The highest frequencies of transduction occurred when the target hematopoietic tissues were infected by cocultivation with a virus-producing line. Cocultivation routinely gave 5–50% G418-resistant (G418^R) cells. The optimal duration of cocultivation depends on the tissue being infected. For fresh bone marrow cells 24 hr of cocultivation is sufficient, but cells from long-term bone marrow culture and splenic lymphocytes require 48 hr for maximum levels of infection (unpublished results).

(*ii*) The number of virus-producing cells present during cocultivation can markedly influence the number of infected cells. In all experiments 5 × 10^5 virus-producing fibroblasts were seeded into 100-mm dishes and allowed to grow overnight. The bone marrow cells (10^7 cells per 100-mm dish) were added to fibroblast cultures that were exposed to 15–20 Gy of radiation to eliminate the accidental transfer of proliferating fibroblasts into subsequent assays of hematopoietic cells. Irradiation had no detectable effect on virus production during the 48-hr period after irradiation (data not shown).

(*iii*) Additional enrichment for neo virus-containing cells can be obtained by harvesting the bone marrow cells after cocultivation and incubating them for 48 hr in liquid culture medium containing very high concentrations of G418 (2 mg/ml). These preselection conditions often yielded populations where 100% of the cells contained and expressed the *neo* gene.

(*iv*) To enhance survival of myeloid progenitors during cocultivation and preselection, appropriate growth factors obtained from media conditioned by WEHI-3B (21) and 5637 (22) cells were added. The former produce large quantities of interleukin 3 (23) and the latter, a factor that promotes self-renewal of myeloid cells (22).

(*v*) In previous experiments, we used bone marrow cells from mice treated with 5-fluorouracil for 2 days before the experiment (7). Although this procedure results in enrichment for the most primitive stem cells, there is no significant difference in the infection frequency of progenitor cells assayed by *in vitro* culture from normal or 5-fluorouracil-treated donors. Because of the large cell loss associated with 5-fluorouracil treatment, we have used only normal bone marrow cells in these experiments.

RESULTS

Selection of G418^R Hematopoietic Progenitor Cells after Infection with Different neo Vectors. Optimal transduction frequencies were obtained when bone marrow cells were cocultivated for 24 hr with irradiated neo virus-producing fibroblasts in the presence of WEHI-3 and 5637 conditioned media. Bone marrow cells were subsequently preselected in high concentrations of G418 for 48 hr and tested for drug resistance by colony formation in methyl cellulose cultures in the presence or absence of G418. We routinely obtained ≈20% G418^R CFU-GM immediately after cocultivation and close to 100% after preselection in G418 prior to the assay for CFU-GM (Table 1). For bone marrow cells infected with MoTN, almost all the CFU-GM that survived the preselection conditions were G418^R in the colony assay. In contrast, fewer that 0.2% of CFU-GM cocultivated with ψ_2 cells survive preselection conditions, and none of the surviving CFU-GM were G418^R in the colony assay. While the absolute infection frequency varied from experiment to experiment (5–50%), preselection always resulted in a 3- to 5-fold enrichment of CFU-GM.

To estimate the relative levels of expression and the proportion of cells expressing the *neo* gene, the degree of drug resistance of the infected and control progenitor cells was measured quantitatively as the percentage of colony-

Table 1. Effect of preselection on the proportion of G418^R CFU-GM following bone marrow infection with MoTN

Vector	Preselection	CFU-GM, no. per 10^5 bone marrow cells: Cell recovery, no. × 10^{-6}	−G418	+G418	% G418^R CFU-GM
MoTN	None	5.0	625	104	17
	G418	1.3	202	156	77
ψ_2	None	5.0	430	0	0
	G418	0.5	0	0	0

Bone marrow cells (10^7) were cocultivated with irradiated MoTN or ψ_2 cells and incubated for a further 48 hr in G418 at 2 mg/ml (preselection) or liquid culture alone (no preselection). The cells were then plated in methyl cellulose culture in the absence (−) or presence (+) of G418 at 1 mg/ml, and the number of G418^R CFU-GM was quantitated.

forming cells that survived increasing concentrations of G418 in methyl cellulose cultures (data not shown). The slopes of these survival curves and extrapolation of the curve to zero G418 are in agreement with the data in Table 1, indicating that a high proportion of the infected cells, close to 100%, express sufficient levels of the *neo* gene product to survive selection.

Because various promoters and enhancers have markedly different effects in different cells, we tested a number of neo vectors containing different promoters and enhancers. The details of each construct are outlined in Fig. 1. Bone marrow cells infected with each of these vectors produced significant numbers of G418^R colony-forming cells indicating successful gene transfer with all of the vectors (Table 2).

Expression of the *neo* Gene after Direct Infection of Progenitors for Different Lineages. The above results indicated that the neo vectors could transfer and express the *neo* gene at very high efficiency. These initial colony assays were done without erythropoietin in the medium, making it not possible to assess gene expression in progenitors for different myeloid lineages. We, therefore, asked specifically whether the *neo* gene could be expressed at high levels in precursors of different cell lineages. For these experiments we used four of the most efficient vectors screened [MLV-NEO.1(Mo-MuLV), NEOμ, MoTN, and N2] to mediate gene transfer into erythroid, myeloid, and multipotent progenitors. Bone marrow cells were cocultivated with each virus-producing cell line, preselected in G418, and plated in methyl cellulose culture in the presence or absence of G418. Infection with each of the four vectors resulted in high frequencies of G418^R BFU-E, CFU-GM, and CFU-MIX (Table 3). The percentage of G418^R colonies formed by cells infected with various vectors did not vary greatly (41–77%). However, as indicated in Table 3, the size of the G418^R colonies produced by MoTN-infected cells was identical to that of colonies grown in the absence of G418, whereas the clones derived from cells infected with MLV-NEO.1 were significantly smaller. No difference was detected in the individual percentages of G418^R erythroid, granulocyte–macrophage, or multipotent progenitors within a population of cells infected with the same virus. These results indicate that the *neo* gene can be efficiently transferred and expressed in progenitors for several myeloid lineages. Inasmuch as the size of colonies grown in G418 depends on the level of expression of the *neo* gene, these data again indicate variability in the levels of expression from different vectors.

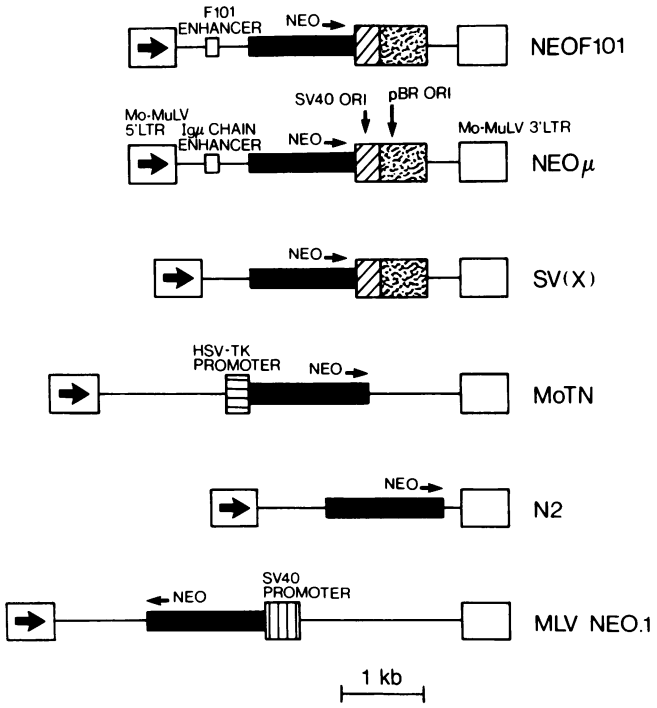

FIG. 1. Schematic diagram of the structure of six retroviral vectors used in this study. HSV, herpes simplex virus.

Table 2. G418^R CFU-GM from bone marrow cells infected with different vectors

Exp.	Vector	Virus titer, cfu/ml	CFU-GM, no. per 10^6 cells −G418	+G418	% G418^R	Colony size
1	MLV-NEO.1-(Mo-MuLV)	5×10^5	1130	1087	96	+
1	SV(X) (ψ_2)	10^5	1340	197	15	+
1	F101 (ψ_2)	5×10^4	1250	58	5	+
1	NEOμ (ψ_2)	5×10^5	1130	486	43	++
2	MLV-NEO.1 (ψ_2)	5×10^5	1560	327	21	+
2	SV(X) (ψ_2)	10^5	530	269	51	+
3	MLV-NEO.1 (ψ_2 and PA12)	5×10^6	1650	670	41	+
3	MoTN (ψ_2)	5×10^6	3120	2830	93	+++
4	N2 (ψ_2)	10^5	625	300	40	++

Bone marrow cells were infected by cocultivation with virus-producing cells, preselected in G418 and plated in methyl cellulose culture in the presence or absence of G418 at 1 mg/ml. cfu, Colony-forming unit. Colony size in G418 was estimated at 30% (+), 50%(++), and 100% relative to colony size in the absence of G418.

Expression of the *neo* Gene in Progenitors Derived from Infected CFU-S. In a study with the MLV-NEO.1 and the NEOμ vectors (7), we reported high efficiency gene transfer in primitive stem cells capable of long-term reconstitution of the hematopoietic system of congenitally anemic W/W^v mice. However, our results (7) and the results of Keller *et al.* (8) indicated that although the original repopulating stem cells expressed the *neo* gene at a detectable level, a smaller proportion of BFU-E, CFU-GM, and CFU-MIX derived from these stem cells expressed the *neo* gene at levels sufficient to confer resistance to G418. However, these experiments were carried out on nonclonal hematopoietic populations derived from long-term reconstituted animals. Therefore, we examined expression of the *neo* gene in clonal populations derived from individual spleen colony-forming cells infected with different retroviral vectors. In the first experiment, CFU-S were examined for their ability to express the *neo* gene. Between 10–30% of the CFU-S survived preselection after cocultivation with cells infected with either MoTN, MLV-NEO.1, or NEOμ vectors, whereas no spleen colonies were observed with bone marrow cells cocultivated with control fibroblasts and preselected in G418. Furthermore, all (26/26) spleen colonies that arose from infected and preselected bone marrow contained the *neo* gene, based on Southern gel analysis (data not shown). Therefore, it can be concluded that all of the spleen colony-forming cells that survived preselection were expressing functional levels of the *neo* gene product.

To determine whether the *neo* gene continues to be expressed as these CFU-S differentiate, individual spleen colonies derived from preselected bone marrow cells were assayed for drug-resistant progenitor cells. Small numbers of bone marrow cells following cocultivation and preselection were injected into lethally irradiated mice. After 12 days, individual spleen colonies were dissected, and each colony was dispersed into a single cell suspension and plated in methyl cellulose cultures in the presence or absence of G418 (24). The results are shown in Table 4. Marked heterogeneity in *neo* gene expression was observed among spleen colonies generated by bone marrow cells infected with different vectors. Some heterogeneity was also observed among spleen colonies derived from cells infected with the same

Table 3. G418^R hemopoietic progenitors of multiple lineages from vector-infected bone marrow cells

Vector	CFU, no. per 10^6 cells: BFU-E −	BFU-E +	CFU-GM −	CFU-GM +	CFU-MIX −	CFU-MIX +	Total −	Total +	% G418^R	Colony size
MLV-NEO.1(Mo-MuLV)	320	190	1100	450	390	100	1810	740	41	+
NEOμ (ψ_2)	160	120	550	380	260	140	970	640	66	++
MoTN (ψ_2)	600	390	1000	830	420	340	2020	1560	77	+++
N2 (ψ_2)	100	20	660	440	70	40	830	500	60	++

Bone marrow cells were infected with the vector indicated, preselected, and plated in the absence (−) and presence (+) of G418 at 1 mg/ml. Symbols are as described in Table 2.

virus. None of the spleen colonies (5 colonies presented in Table 4 and 19 not shown) derived after infection with MLV-NEO.1 contained G418^R progenitors. Two out of four spleen colonies grown from NEOμ-infected cells contained a total of 3% and 42% G418^R progenitors. Interestingly, a difference in the frequency of drug-resistant BFU-E or CFU-MIX as compared to CFU-GM was observed. Colony 3 had 37% G418^R CFU-GM while virtually all BFU-E and CFU-MIX were G418^R. Only 3% of CFU-GM in colony 4 were G418^R; however, if BFU-E and CFU-MIX had shown similar low levels of drug resistance in this colony, we could not have detected them because of the low numbers of progenitors. All four spleen colonies generated by MoTN-infected cells showed G418^R progenitors with a frequency ranging between 9% and 80%.

Expression of the *neo* Gene in Fibroblasts Infected with Different neo Vectors. Since there appeared to be large differences in the level of *neo* gene expression in hematopoietic cells, we also compared the relative levels of *neo* gene expression in rodent fibroblasts infected with these retroviral vectors. The activity of the *neo* gene product in infected Rat-2 cells was assayed directly by the neomycin phosphotransferase enzymatic assay (Fig. 2). In contrast to the results observed with hematopoietic progenitor cells, MLV-NEO.1 produced the largest amount of enzymatic activity in Rat-2 cells while the level of activity in MoTN-infected cells was lower by at least a factor of 10–50. The level of enzyme activity in N2- and NEOμ-infected cells was 30–50% of the MLV-NEO.1 activity.

Table 4. G418^R hemopoietic progenitor cells from individual spleen colonies

Vector	Spleen colony	CFU, no. per spleen colony: BFU-E −	BFU-E +	CFU-GM −	CFU-GM +	CFU-MIX −	CFU-MIX +	Total −	Total +
NEOμ (ψ_2)	1	28	0	56	0	0	0	84	0
	2	0	0	8	0	0	0	8	0
	3	64	53	605	223	11	11	680	287
	4	31	0	289	10	49	0	369	10
MLV-NEO.1-(Mo-MuLV)*	5	0	0	1000	0	0	0	1000	0
	6	0	0	31	0	0	0	31	0
	7	0	0	0	0	0	0	0	0
	8	26	0	462	0	4	0	492	0
	9	101	0	331	0	58	0	490	0
MoTN (ψ_2)	10	†						70	26
	11							140	88
	12							663	59
	13							55	44

Cells obtained from individual spleen colonies of mice 12 days after reconstitution with NEOμ (ψ_2)-, MLV-NEO.1(Mo-MuLV)-, or MoTN (ψ_2)-infected bone marrow that had been preselected were plated in methylcellulose cultures in the presence (+) or absence (−) of G418 at 1 mg/ml.

*Only data from 5/24 spleen colonies are shown. The other 19 spleen colonies did not contain any G418^R progenitor cells.

†Only the total number of colonies was determined.

DISCUSSION

Gene transfer with retrovirus vectors is a highly efficient process. Immediately after cocultivation, between 5% and 50% of hematopoietic progenitors are resistant to selective concentrations of G418 in *in vitro* colony assays. Analysis of the survival curves of infected progenitors indicates that an even larger proportion of cells are infected but that many of the cells fail to express high enough levels of the *neo* gene product to survive a selective concentration of G418. Analysis of spleen colonies shows that ≈10% of CFU-S contain the *neo* gene following cocultivation (6, 7). The proportion of cells that express the *neo* gene can be enriched by exposing the infected cells to high concentrations of G418 for 48 hr. In this way, populations of bone marrow progenitor cells can be obtained with 60–100% of the surviving *in vitro* progenitor cells resistant to G418 and 100% of surviving CFU-S carrying the *neo* gene (7).

Six different neo vectors, differing in orientation as well as in the promoters and enhancers used to activate and regulate *neo* gene transcription, were analyzed in the present study. With all six neo vectors, we observed transfer and expression of the *neo* gene in multiple hematopoietic lineages. However, the level of *neo* gene expression, as judged by the frequency of drug-resistant colony-forming cells and the size of the hematopoietic colonies growing in drug, varied significantly among the different vectors. NEOμ-, MoTN-, and N2-infected progenitors produced larger colonies in G418 than

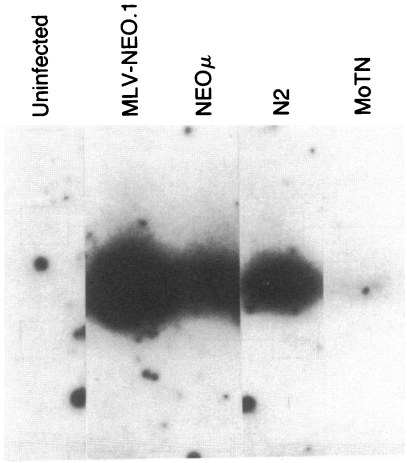

FIG. 2. Neomycin phosphotransferase activity in rat fibroblasts infected with neo vectors. Rat-2 cells were infected with the retroviral vectors indicated and selected in G418. The G418^R cells were disrupted by sonication, and the level of neomycin phosphotransferase activity was assayed.

did MLV-NEO.1-infected cells. Since the titer on fibroblasts of all the neo vector-producing cell lines was similar, these variations are likely to be due to different levels of *neo* gene transcription in these progenitor cells.

Expression of the *neo* gene was also measured in the multipotent progenitor CFU-S, as well as the progenitors that arise as CFU-S differentiate. No spleen colonies are produced when uninfected bone marrow is preselected before assaying for CFU-S. In contrast, all spleen colonies that are generated from infected bone marrow following preselection contain the *neo* gene, based on Southern analysis (7). These results indicate significant levels of *neo* gene expression, at least transiently, in CFU-S following cocultivation. However, progenitors within individual spleen colonies show a large degree of heterogeneity in *neo* gene expression despite the fact all the cells in a single spleen colony contain the gene integrated at the same genomic site. No $G418^R$ colony-forming cells could be detected in the spleen colonies arising from MLV-NEO.1-infected CFU-S, whereas all the spleen colonies generated from MoTN-infected CFU-S contained significant numbers of $G418^R$ progenitors.

Several different mechanisms may account for the variability in *neo* gene expression in the progeny of stem cells infected with different retroviral vectors. First, the heterogeneity in gene expression between various spleen colonies may reflect intrinsic differences in transcriptional activity at different chromosomal sites of vector integration. Second, the modulation in *neo* gene expression reported here may also reflect changes in transcriptional activity at particular chromosomal sites of integration as stem cells differentiate.

Third, differences in gene expression clearly reflect the particular promoter used to activate the *neo* gene in various vectors. For example, all the spleen colonies generated from MoTN-infected cells expressed the *neo* gene, whereas spleen colonies generated with MLV-NEO.1 did not. These data and the fact that MLV-NEO.1-infected fibroblasts produce high levels of neomycin phosphotransferase suggests the SV40 promoter may be less active in hematopoietic progenitor cells. Furthermore, Williams *et al.* (25) have also reported their inability to detect human adenosine deaminase in spleen colonies derived from CFU-S infected with retrovirus vectors carrying the human adenosine deaminase gene expressed from the SV40 promoters.

Fourth, the observations described here are strikingly similar to observations made after infection of preimplantation mouse embryos and teratocarcinoma cells with retroviruses and retrovirus vectors. Jaenisch and his colleagues (26) have shown that Mo-MuLV is generally not expressed in transgenic mice derived from infection of preimplantation embryos. Similarly the retroviral genome is inefficiently expressed in undifferentiated embryonal carcinoma cells (27–29). Experiments carried out with retrovirus vectors have also demonstrated inactivity of the viral LTR following infection of preimplantation embryos (30) and teratocarcinoma cell lines (31), and we have shown that the SV40 early region promoter is inactive in transgenic mice generated by infection of early embryos with MLV-NEO.1 (32). Gorman *et al.* (33) have suggested that there may be trans-acting negative regulatory factors in these undifferentiated cell types that specifically inhibit transcription from the LTR and SV40 early region promoters.

Although there is no definitive evidence that the same molecular processes that down-regulate retroviral gene expression in early mouse embryos and embryonal carcinoma cells also operate in hematopoietic stem cells, the results presented here indicate that high levels of expression can be attained in hematopoietic stem cells with retroviral vectors containing the herpes virus *tk* promoter. Consistent with this observation, Stewart *et al.* (34) have reported expression of the *neo* gene in chimeric mice derived from embryonic stem cells infected with a retrovirus vector that, like MoTN, includes the herpes *tk* promoter.

Our results show that a foreign gene can be expressed to biologically significant levels in hematopoietic stem cells and their progeny during short-term *in vivo* reconstitution and *in vitro* plating. We have also observed high levels of $G418^R$ progenitor cells in mice reconstituted 8 months earlier with a stem cell clone infected with MoTN. These results suggest that foreign genes continue to be expressed *in vivo* for extended periods of time following long-term reconstitution with stem cell clones infected with appropriate retroviral vectors.

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1. Till, J. E. & McCulloch, E. A. (1980) *Biochim. Biophys. Acta* **605**, 431–459.
2. Ogawa, M., Porter, P. N. & Nohohota, T. (1983) *Blood* **61**, 823–829.
3. Dick, J. E., Magli, M. C., Phillips, R. A. & Bernstein, A. (1986) *Trends Genet.* **2**, 165–170.
4. Joyner, A., Keller, G., Phillips, R. A. & Bernstein, A. (1983) *Nature (London)* **305**, 206–208.
5. Miller, A., Eckner, R., Jolly, D., Friedman, T. & Verma, I. (1984) *Science* **225**, 630–632.
6. Williams, D. A., Lemischka, I. R., Nathans, D. G. & Mulligan, R. C. (1984) *Nature (London)* **310**, 476–480.
7. Dick, J. E., Magli, M. C., Huszar, D. H., Phillips, R. A. & Bernstein, A. (1985) *Cell* **42**, 71–79.
8. Keller, G., Paige, C., Gilboa, E. & Wagner, E. F. (1985) *Nature (London)* **318**, 149–154.
9. Eglitis, M., Kantoff, P., Gilboa, E. & Anderson, W. F. (1985) *Science* **230**, 1395–1398.
10. Hock, R. A. & Miller, A. D. (1986) *Nature (London)* **320**, 275–277.
11. Lemischka, I. R., Raulet, D. H. & Mulligan, R. C. (1986) *Cell* **45**, 917–927.
12. Iscove, N. N., Guilbert, L. J. & Weyman, C. (1980) *Exp. Cell Res.* **126**, 121–126.
13. Till, J. E. & McCulloch, E. A. (1961) *Radiat. Res.* **14**, 213–222.
14. Joyner, A. & Bernstein, A. (1983) *Mol. Cell. Biol.* **3**, 2180–2190.
15. Cepko, C., Roberts, B. & Mulligan, R. (1984) *Cell* **37**, 1053–1062.
16. Levine, A. J. (1982) *Curr. Top. Microbiol. Immunol.* **101**, 1–30.
17. Mann, R., Mulligan, R. C. & Baltimore, D. (1983) *Cell* **38**, 153–159.
18. Miller, A., Law, M. D. & Verma, I. (1984) *Mol. Cell. Biol.* **5**, 431–437.
19. Miller, A. D., Trauber, D. R. & Buttimore, C. (1986) *Somatic Cell Mol. Genet.* **12**, 175–183.
20. Reiss, B., Sprengel, R., Will, H. & Scholler, H. (1984) *Gene* **30**, 211–218.
21. Ralph, P. & Nakoinz, I. (1977) *Cancer Res.* **37**, 546–550.
22. Hoang, T. & McCulloch, E. A. (1985) *Blood* **66**, 748–751.
23. Ihle, S. N., Keller, J., Henderson, L., Klein, F. & Palaszynski, E. (1982) *J. Immunol.* **123**, 2431–2436.
24. Magli, M. C., Iscove, N. N. & Odartchenko, N. (1982) *Nature (London)* **295**, 527–529.
25. Williams, D. A., Orkin, S. H. & Mulligan, R. C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2566–2570.
26. Stuhlmann, H., Jahner, D. & Jaenisch, R. (1981) *Cell* **26**, 221–232.
27. Stewart, C. L., Stuhlmann, H., Jahner, D. & Jaenisch, R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4098–4102.
28. Niwa, O., Yokota, Y., Ishida, H. & Sugahara, T. (1983) *Cell* **32**, 1105–1113.
29. Gautsch, J. W. & Wilson, M. C. (1983) *Nature (London)* **301**, 32–35.
30. Jahner, D., Haase, K., Mulligan, R. & Jaenisch, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6927–6931.
31. Linney, E., Davis, B., Overhauser, J., Chao, E. & Fan, H. (1984) *Nature (London)* **308**, 470–472.
32. Huszar, D., Balling, R., Kothary, R., Magli, M. C., Hozumi, N., Rossant, J. & Bernstein, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8587–8591.
33. Gorman, C. M., Rigby, P. W. S. & Lane, D. P. (1985) *Cell* **42**, 519–526.
34. Stewart, C. L., Vanek, M. & Wagner, E. F. (1985) *EMBO J.* **13**, 3701–3709.